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Testate amoebae in pollen slides.

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ABSTRACT

Among the non-pollen micro-fossils commonly encountered in Quaternary sediment samples prepared for pollen analysis are many shells of testate amoebae. Testate amoebae are eukaryotic micro-organisms which are increasingly used in ecological and palaeoecological studies, particularly as indicators of hydrological change in *Sphagnum*-dominated peatlands. In this study we address the extent to which testate amoebae are used in palynological research, the key challenges to more

widespread use, and the extent to which ecological information is retained in the testate amoeba assemblages of standard palynological slides. To achieve this we review the literature on the use of testate amoebae in palynology, compare testate amoeba records produced by palynological and water-based preparation methods and carry out simulations using previously-derived datasets. Our results show that testate amoebae are widely encountered in Quaternary palynological studies, primarily in peatlands, but the information which they can provide is undermined by limited taxonomic knowledge. Many taxa are destroyed in pollen preparations, but for taxa that are retained patterns of abundance parallel those determined using water-based preparation methods. Although the loss of sensitive taxa limits the ecological information contained in testate amoeba assemblages the information preserved is likely to be useful in a multiproxy approach to palaeoenvironmental reconstruction. To help improve taxonomic awareness and encourage the use of testate amoebae in palynology we present a basic introduction to testate amoeba taxonomy and a guide to the taxonomic literature.

KEYWORDS: Testate amoebae; Non-pollen palynomorphs; Pollen; Palynology; Protists

INTRODUCTION

A variety of Quaternary microfossils other than pollen are commonly found in slides prepared for pollen analysis and are collectively termed non-pollen palynomorphs (NPPs). NPPs include the sub-fossil remains of a large variety of organisms with a diversity of ecological niches which may be sensitive to many environmental gradients and changes to which the pollen record is relatively unresponsive. For instance in palaeolimnology while pollen is predominantly allogenic many NPPs are autogenic, formed by aquatic organisms such as algae, rotifers and cyanobacteria and may thus record information on changes in the aquatic ecosystem to which the pollen record is less sensitive. Specific groups of NPPs may provide information on human impacts, ecosystem development and fire-history among many other environmental changes (van Geel 2001). Palaeoecological studies are increasingly recognising that non-pollen palynomorphs can provide useful information which complements that from pollen, at a modest cost in additional effort (e.g. Mighall et al. 2006; Riera et al. 2006).

Among the microfossils grouped as non-pollen palynomorphs are testate amoebae. Testate amoebae are a group of eukaryotic microorganisms characterised by a test: a decay-resistant shell enclosing the cytoplasm, some of which survive pollen preparations (Hendon & Charman 1997). Testate amoebae are present in numerous environments including soils, lakes, wetlands, coastal environments and even glaciers (Charman 2001; Mitchell et al. 2008a; Santibanez et al. 2008). Around 2500 species have been described and, with the limited attention paid to testate amoebae in many environments and regions and the presence of many cryptic and pseudo-cryptic taxa (Lara et al. 2008, 2011; Heger et al. 2011) this figure is most likely conservative (Mitchell et al. 2008a).

Testate amoebae are among the most morphologically variable of all micro-fossils studied by palaeoecologists, ranging in size by two orders of magnitude (ca. 4 to 400µm) with a wide range of body plans and a variety of often highly-distinctive surface ornamentation (Fig. 1). Tests are constructed of

endogenous plates (idiosomes), endogenous secretions, or agglutinated environmental particles (xenosomes). The chemical composition of tests is variable including species with largely siliceous, calcareous and organic tests (Meisterfeld 2002a, b). Tests have been extracted and studied by palaeoecologists in bogs, lakes and saltmarshes (Charman 2001; Roe et al. 2002; Wall et al. 2010). The aim of most focussed palaeoecological studies of testate amoebae has been the reconstruction of hydrological variability to investigate climatic change using peatland records and sea-level change using coastal sediment records (Charman 2001). Many transfer function models have been produced to quantify these changes (Charman 2001; Mitchell et al. 2008a).

Tests of many testate amoeba species are relatively sensitive to chemical treatments and it has been known for decades that while some tests survive pollen preparations many taxa are not preserved (e.g. Aaby 1976). Studies specifically directed at testate amoebae have therefore generally used less harsh methods. The most common preparation method involves suspension in water, boiling and then sieving at around 15 µm and 300 µm and mounting in glycerol or water (Hendon & Charman 1997; Booth et al. 2010/2011). Testate amoebae have been included among non-pollen palynomorphs in many palynological studies. However, as we demonstrate here, use of testate amoebae is inconsistent and compromised by lack of taxonomic knowledge.

In this study we catalogue the current use of testate amoebae in Quaternary palynology, highlight priorities for future study and investigate new research avenues. We first review the literature to gain an understanding of the use of testate amoebae in palynological studies. Subsequently we present a direct comparison of palaeoecological testate amoeba records produced by palynological and water-based preparations methods. Finally, we apply transfer functions to simulated datasets to determine the extent to which palaeoecological information is preserved and whether quantitative

81 palaeoecological reconstruction may be possible from the denuded assemblages in palynological
82 studies.

83 METHODS

84 Current use of testate amoebae in palynology

85 To assess the current state of the art in the use of testate amoebae as NPPs we conducted a
86 literature review. We searched *Google Scholar* in May 2010 for combination of the terms 'non-pollen
87 palynomorphs', 'pollen' or 'palynology' with 'testate amoebae' (and obvious synonyms: arcellaceans,
88 testate rhizopods etc) supplemented with any further studies with which we were acquainted. We
89 scanned all identified papers for testate amoeba taxa listed in the text or included in biostratigraphic
90 diagrams and noted how the testate amoebae were used in the palaeoenvironmental interpretation, if
91 at all.

92 Palynological vs conventional testate amoeba analysis

93 To compare community composition and temporal trends in testate amoebae between
94 palynological and water-based preparations we present both types of records through the length of the
95 same peat core from Mauntschas in the Eastern Alps of Switzerland. Lamentowicz et al. (2010) have
96 presented a detailed water-based testate amoeba diagram from this transitional mire with very high
97 resolution and marked changes in community composition. Here we present testate amoeba data
98 obtained in the course of palynological analysis of the core (the subject of a forthcoming paper). Water-
99 based testate amoeba samples were prepared using the wet sieving method of Hendon & Charman
100 (1997), palynological samples were prepared using KOH and acetolysis (Kamenik et al. 2009). In counting
101 testate amoebae in the pollen slides particular attention was paid to achieving high count totals; mean
102 count total was 69, approximately half of the total count of 150 achieved in the water-based analysis

103 although with greater variability. To investigate how the palynological testate amoeba record compares
104 to the water-based record one of us with no prior acquaintance with work at this site (RJP) attempted a
105 qualitative reconstruction of wetness changes based on the palynological testate amoeba record.

106 Quantitative environmental reconstruction from palynological testate amoeba analysis?

107 Finally, we simulated by artificial data manipulation the conditions of palynological testate
108 amoeba analysis using datasets derived from water-based preparations to determine the extent to
109 which these conditions alter quantitative palaeoecological reconstruction. These simulations have two
110 purposes: to test whether palaeoehydrological information is adequately preserved in the reduced data
111 set and to test whether the transfer function approach commonly used in palaeoecology is applicable to
112 palynological testate amoeba records. Is a real palaeoecological signal preserved or do differential
113 decomposition and low counts so skew the results that no valid quantitative reconstruction is possible?
114 We manipulated datasets (Table 1) using experimental scenarios designed to approximate conditions of
115 palynological testate amoeba analysis and then applied transfer functions based on (unmodified)
116 modern training sets to reconstruct changes in peatland depth to water table over time. The
117 palaeoecological datasets and their respective transfer functions are: 1. 'Site DLB', a peatland in sub-
118 Arctic Alaska (Payne *et al.* unpublished, Alaska transfer function: Payne *et al.* 2006); 2. Praz-Rodet, a
119 peatland in Switzerland (Mitchell *et al.* 2001, Jura transfer function: Mitchell *et al.* 1999, 2001) and 3.
120 Jelenia Wyspa, a peatland in Poland (Lamentowicz *et al.* 2007, Poland transfer function: Lamentowicz *et*
121 *al.* 2008). All studies used variants of the wet-sieving preparation method of Hendon and Charman
122 (1997).

123 Our experimental scenarios included three types of data manipulation:

1. As tests of many taxa do not survive pollen preparations, our simulations only included testate amoeba taxa which have been found in palynological studies (shown by our literature review) and two additional taxa which may be preserved: *Arcella catinus* and *Cryptodifflugia oviformis*.
2. As even for taxa which do survive pollen preparations not all individual tests survive (cf. Swindles & Roe 2007) we down-weighted sensitive taxa. Hendon and Charman (1997) have experimentally investigated the preservation of testate amoebae in pollen preparations. **I**n our simulations we took average losses from major taxa (>1% total) in all palynological preparations in this study (in one case excluding an apparent increase) and manipulated our palaeoecological datasets accordingly (*cf*. Mitchell et al. 2008b). Data was only available for a limited range of taxa, other taxa were left unchanged.
3. As the count of testate amoebae in palynological studies is generally small we simulated reduced counts. In our experiments we simulated counts of 20 tests, a total which can often be reached with little additional counting effort in palynological studies. Reduced counts were simulated by random selection of individuals with replacement, each individual having an equal probability of selection (Payne & Mitchell 2009).

We carried out four sets of experiments using these scenarios: 1) selected taxa only, 2) selected taxa and downweighting of sensitive taxa, 3) selected taxa with reduced count, and 4) selected taxa, downweighting of sensitive taxa and reduced count. All three palaeoecological datasets were manipulated to reflect these changes and percentages calculated. Applicable transfer functions were then applied and depth to water table reconstructed using C^2 vers. 1.4 (Juggins 2003). Reconstructed values are termed testate amoeba inferred depth to water table (TI-DWT).

RESULTS and DISCUSSION

Current use of testate amoebae in palynology

We identified 51 palynological studies which have included testate amoebae as NPPs, although we suspect that the real total is much greater (Table 2). The most frequent taxa were *Amphitrema (=Archerella) flavum* (22 studies), undifferentiated *Arcella* species (20 studies) and *Assulina seminulum* (12 studies). At least 20 species are represented with *Arcella* the best represented genus (8 species). The list includes some relatively rare taxa such as *Arcella stellaris* and *Phryganella nidulus*, although most taxa are common. The highest numbers of taxa were identified in studies which aimed for a higher count (e.g. Muller et al. 2003) or included trained testate amoeba analysts (e.g. Wheeler et al. 2010). The vast majority of studies are from peats or similar organic contexts, a smaller number from lacustrine sediments, and only a few from archaeological contexts (e.g. Kvavadze et al. 2008). The studies are widely dispersed but predominantly originate from Europe, and particularly the Netherlands, with notably few examples from North America. Almost all studies identifying testate amoebae are Holocene.

Some studies have presented curves for total testate amoebae, while many have only been identified to genus level. Some studies present illustrations of 'unknown microfossils' or 'unknown testate amoebae', which in our view are easily identifiable at least to genus level. This lack of taxonomic detail clearly limits the potential ecological information. For instance, Borromei et al. (2010) consider *Arcella* (undiff.) to indicate shallow, meso-eutrophic or mesotrophic freshwater pools. However, *Arcella* is a large genus including taxa found in both oligotrophic and brackish conditions, which makes such an assertion difficult to support. Miehe et al. (2009) interpret the presence of undifferentiated testate amoebae as 'nutrient enrichment by soil erosion and faeces'. This is difficult to support, given the abundance of testate amoebae in oligotrophic habitats and the deleterious impact of experimental nutrient addition (e.g. Gilbert et al. 1998). Although such inferences are often made on a multiproxy basis it is clear that if these tests were identified to a higher taxonomic level, the potential ecological information they could provide would be vastly increased.

Most tests identified are composed of organic secretions (e.g. *Archerella, Arcella, Hyalosphenia*) or organic-coated idiosomes (*Assulina*). These are generally considered the most robust to chemical treatment. Some xenosome tests are reasonably well represented, including *Centropyxis* and *Phryganella* however many common xenosome genera are poorly represented, with only one record of the abundant genus *Difflugia* and one of *Heleopera*. Genera with tests constructed of idiosomes without organic coating are absent apart from a single record of *Corythion-Trinema* type (Yeloff et al. 2007); the latter probably reflects the unusual preservation conditions associated with removal of silica limitation by deposition of volcanic ash. Studies have used a variety of pollen preparation methodologies but it is not clear from the results whether some methods retain more tests than others. Although beyond the scope of the current paper it is also worth noting that testate amoebae have been recorded in some macrofossil studies (e.g. van der Linden et al. 2008b). More frequent and reliable identification of testate amoebae in these studies would be beneficial.

Palynological vs water-based testate amoeba analysis

In Mauntschas, testate amoeba concentrations and accumulation rates (Fig. 2) for palynological preparations are considerably lower than for water-based methods, varying by taxon and depth but averaging less than 10%. Although there may be real reasons for differences in concentration between different samples from the same depths much the most probable explanation is that many tests are destroyed or lost in preparation. There is a large degree of commonality between the palynological and water-based records for the four testate amoeba taxa which are found in both. *Archerella flavum* is only found in the lower part of the core for both records with similar peaks at 57, 60 and 65 cm (although a peak in abundance at 58 cm is absent in the palynological record). Most *Arcella* tests are found between 35 and 60 cm in both records, with a major peak in both at 57 cm. *Assulina muscorum* shows a pattern of peaks and troughs that is similar between the two records but has considerable differences in the

magnitude of change, notably in the major peak at 9 cm in the water-based record. Peaks at 56 and 66 cm appear less marked in the water-based record. *Assulina seminulum* has very similar peaks and troughs but the magnitude of change in the water-based record is distorted by a large peak at 9 cm. In general there are very similar patterns of change between the two records, and though the magnitude of change often differs, the direction of change is mostly the same. We conclude that even for taxa which survive pollen preparations a majority of individuals is not retained. Despite this, the pattern of change is reasonably intact, so that the ecological information of these taxa is retained.

Fig. 3 compares a qualitative reconstruction of hydrological change based on the palynological record with the quantitative reconstruction based on water-based preparation presented in the original paper (Lamentowicz et al. 2010). The palynologically prepared record suggested a drier phase in the upper 30 cm on the basis of abundance of *Assulina muscorum* and *Assulina seminulum*, which is confirmed by the transfer function results. From 50-68 cm a phase of alternating water tables was suggested with distinct peaks in the hydrophilic species *Archerella flavum* at 57, 61 and 66 cm assumed to represent short-lived wetter-periods. This inference is not supported by the transfer function results, which rather suggest consistently wetter conditions. For much of the core there were very few preserved tests and it was not possible to make any palaeoenvironmental inference. Although there is similarity in the pattern of change in the retained taxa, the loss of most of the assemblage prevents an accurate environmental reconstruction.

<u>Quantitative environmental reconstruction from palynological testate amoeba analysis?</u>

Results of the transfer function simulations are shown in Figs. 4-6. For site DLB the most pronounced features of the original reconstruction are wet phases at the base of the sequence, from 26-29 cm and 53-56 cm. These features are still apparent when only using selected taxa, although a more minor trough at 38 cm is exaggerated. When taxa are also down-weighted using scenarios from Hendon

& Charman (1997), the original pattern is largely destroyed and a sequence of phases appears that is not present in the original reconstruction. With reduced counts reconstructions bear even less similarity to the original reconstructions, particularly when considering individual results (not presented). For Jelenia Wyspa (Fig. 5) the most distinct features of the original reconstruction are a sequence of peaks between 25 and 35 cm and a high plateau between 50 and 65 cm. When using only taxa which survive pollen preparations the sequence of peaks is still present while the plateau is largely absent, and the same is the case when sensitive taxa are also downweighted. If the effective count size is also reduced, considerable variability appears in which many reconstructions do not include features present in the original and vice versa. For Praz Rodet (Fig. 6) the use of selected taxa only changes the shape of the reconstruction in the upper 60 cm, introducing a period of increasing values. A peak at 246 cm is reduced in magnitude while a period of high values from 190 to 202 cm is stretched. Downweighting selected taxa or reducing the count produces further minor deviations from the original reconstruction, but combining both these treatments produces reconstructions which bear little similarity to the original reconstruction.

Taken overall, exclusion of taxa which do not survive pollen preparations, downweighting of taxa where only some individuals survive, and reduction of count-totals successively destroys the palaeoecological 'signal' apparent in reconstructions based on water-based preparations. While the low count-total can be addressed in palynological studies by simply counting more individuals (e.g. Muller et al. 2003), the loss of tests cannot. We conclude that if transfer functions are applied to palaeoecological data derived from pollen preparations, there is a high probability of producing reconstructions which are inprecise or erroneous.

<u>The potential of testate amoebae in NPP studies</u>

Testate amoebae are widely, and increasingly, included among the diverse range of microfossils counted as non-pollen palynomorphs. The value of these records is currently limited by poor taxonomic resolution and possibly by taxonomic inaccuracy. In this paper we show that the curves for testate amoebae derived from palynological records may closely mirror those from water-based testate amoeba preparations. Although the loss of most of the assemblage under-mines both qualitative and quantitative reconstructions of environmental change based on testate amoebae alone we suggest that testate amoebae are still of use to palynologists. The retention of ecological information in the taxa which do survive pollen preparations suggests that testate amoebae may be a useful component of a multiproxy approach incorporating other NPPs and other methods. In many environments testate amoebae are likely to compose a large proportion of the total NPP assemblage and our knowledge of the ecology of some taxa is such that they may provide useful additional information. Plotting ordination scores may be a simple way to integrate this information (Rull et al. 2008). By far the best known aspect of testate amoeba ecology is their hydrological preferences and their predominant use in palaeoecology has been for the reconstruction of hydrological change. However there is an increasingly long list of environmental changes to which testate amoebae have been shown to be sensitive including acidification, eutrophication (Gilbert et al. 1998) and pollution by sulphur (Payne et al. 2010), nitrogen (Mitchell 2004) and metals (Asada & Warner 2009). Although the species response to these changes is not always well-characterised this situation is rapidly improving. As well as aiding palaeoenvironmental reconstruction the more frequent and accurate identification of testate amoebae in palynology would also help improve our knowledge of testate amoeba ecology. The preservation of testate amoebae in palynological samples may well vary according to palynological preparation technique. We are not currently able to recommend a preparation method which will both produce high quality pollen slides and also optimise preservation of testate amoebae. Certainly the use of strong acids (HF) is likely to be very destructive for tests.

Suggestions for study of testate amoebae in palynological research

The use of testate amoebae in palynology is limited by taxonomic knowledge. Unfortunately there is no single modern, comprehensive guide to testate amoeba taxonomy which can be recommended for palynologists. The most widely used guide for palaeoecologists is that of Charman et al. (2000). This useful guide is readily and cheaply available and covers the most common taxa in peatlands. However, the guide takes a rather conservative approach with many broad groupings which do not find favour with all taxonomists, and it does not attempt to include taxa absent in oligotrophic peatlands. The most comprehensive modern guide is that of Mazei and Tsyganov (2006), which includes the majority of known taxa, but is only available in Russian. Ogden & Hedley (1980) is a useful guide to a limited range of taxa and has beautiful SEM images, but is out of print. Other taxonomic guides which may be of use to palynologists include: Grospietsch (1958), Harnisch (1958), Corbet (1973), Ellison and Ogden (1987), Clarke (2003), Warner (1990) for general introduction and Ogden (1983) for *Difflugia*, Deflandre (1936) for *Nebela*, Deflandre (1928) for *Arcella*, Deflandre (1929) for *Centropyxis*, and Grospietsch (1965) for *Hyalosphenia*.

In Appendix One we set out a basic identification guide to some common testate amoeba taxa for palynologists. We base our list on the taxa identified in our literature review supplemented with taxa which we believe might be preserved. Among the many hundred species of testate amoeba that could conceivably be found in pollen slides, our list is biased towards peatland taxa, reflecting the greater abundance of NPP studies in this environment. It will, however, be wise to cross-check identifications with more detailed taxonomic guides. We avoid specialist terminology and keep identification criteria as simple and straightforward as possible, and hope that the result is of practical use to palynologists.

CONCLUSIONS

Testate amoebae are widely present in Quaternary palynological samplesand constitute an under-utilised source of palaeoecological information. The testate amoeba assemblage in such slides is extensively denuded such that it would be unwise to rely on testate amoebae alone for environmental reconstruction. However, the records for taxa which do survive mirror unbiased records produced by water-based preparation methods. Testate amoebae may therefore provide useful additional information in a multiproxy approach. Current use of testate amoebae is undermined by lack of awareness and limited taxonomic knowledge. As a step towards remedying this situation we provide a basic taxonomic guide and suggestions for further reading.

296

297 FIGURES and TABLES

298 Table 1. Details of palaeoecological records used in this study. For full details of site and methods see

299 original publications.

300 Table 2. Testate amoeba taxa recorded in 51 published Quaternary NPP studies.

301 Fig. 1. Photomicrographs of selected testate amoeba taxa which may survive pollen preparations. 1-2.

302 *Arcella gibbosa*, 3. *Arcella hemispherica*, 4. *Arcella catinus*, 5. *Arcella discoides*, 6. *Arcella vulgaris*, 7. *A.*

303 *dentata*, 8. *Centropyxis constricta*, 9. *C. laevigata*, 10. *C. ecornis*, 11. *C. platystoma*, 12. *C. aerophila* , 13.

304 *C. aculeata*, 14. *Trigonopyxis arcula*, 15. *Bullinularia indica*, 16. *Nebela langeniformis*, 17. *N. carinata*, 18.

305 *N. penardiana*, 19. *N. militaris*, 20. *Physochila griseola*, 21. *Argynnia dentistoma*, 22. *Heleopera rosea*,

306 23. *Difflugia leidyi*, 24. *D. pulex*, 25. *Hyalosphenia elegans*, 26. *H. subflava*, 27. *H. papilio*, 28. *Archerella*

307 *flavum*, 29. *Amphitrema wrightianum*, 30. *Assulina scandinavica*, 31. *A. seminulum*, 32. *A. muscorum*,

308 33. *Phryganella acropodia*.

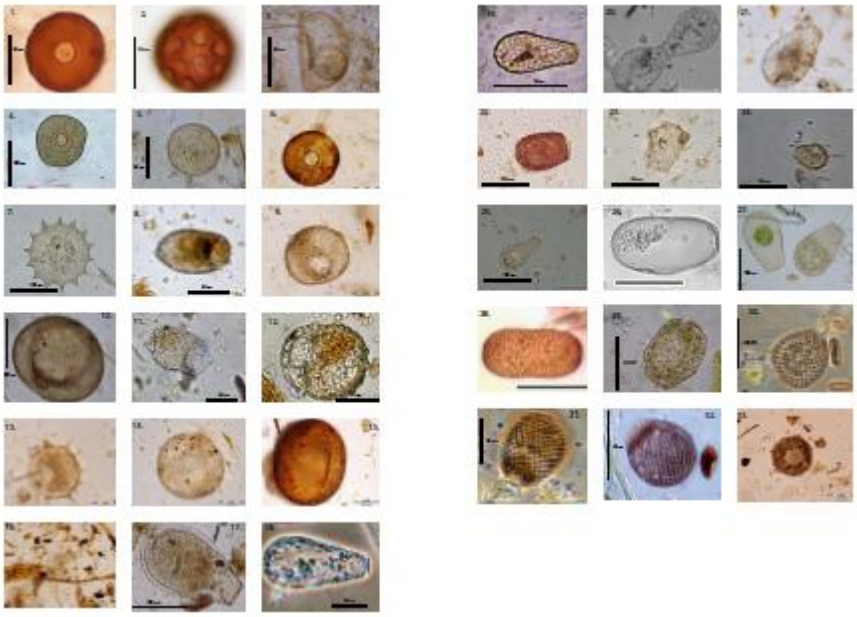

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Fig. 2. Water-based (white points) and palynologically based (black points) testate amoeba records from Mauntschas, Switzerland, also showing R^2 based on points common to both profiles.

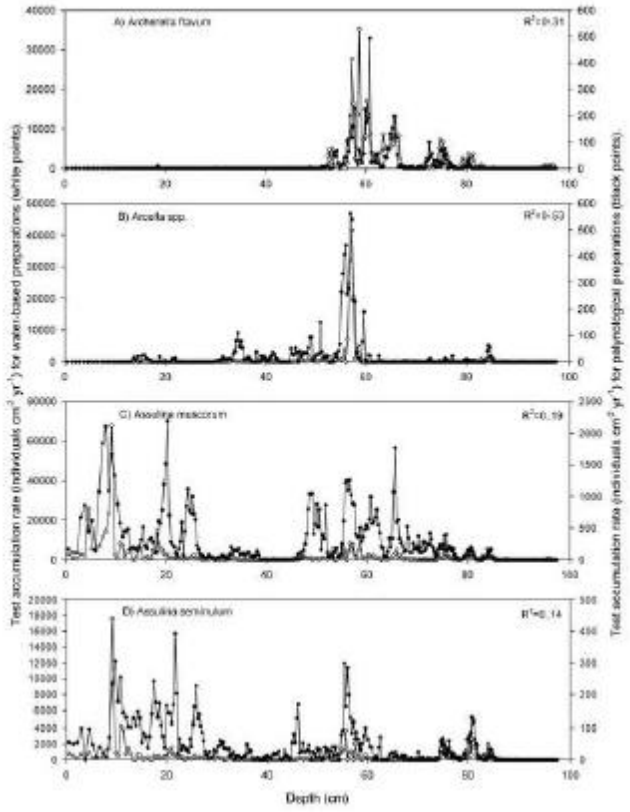


Fig. 3. Comparison of qualitative hydrological reconstruction using testate amoeba NPP record (vertical bars, red=dry-phase, blue=wet-phase, grey=insufficient information) with quantitative transfer-function-derived reconstruction of Lamentowicz et al. (2010).

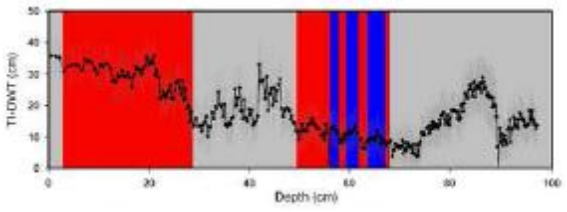


Fig. 4. Simulation results for site DLB (Alaska, USA) showing a) original hydrological reconstruction, b) simulation using only testate amoeba taxa which survive pollen preparations (Table 2), c) simulation

using only taxa which survive pollen preparations with down-weighting of surviving taxa based on results of Hendon & Charman (1997), d) simulation using only taxa which survive pollen preparations with simulated count-total reduced to 20 (20 cycles of random-selection), e) simulation using only taxa which survive pollen preparation, down-weighting of surviving taxa and reduced count-total as above.

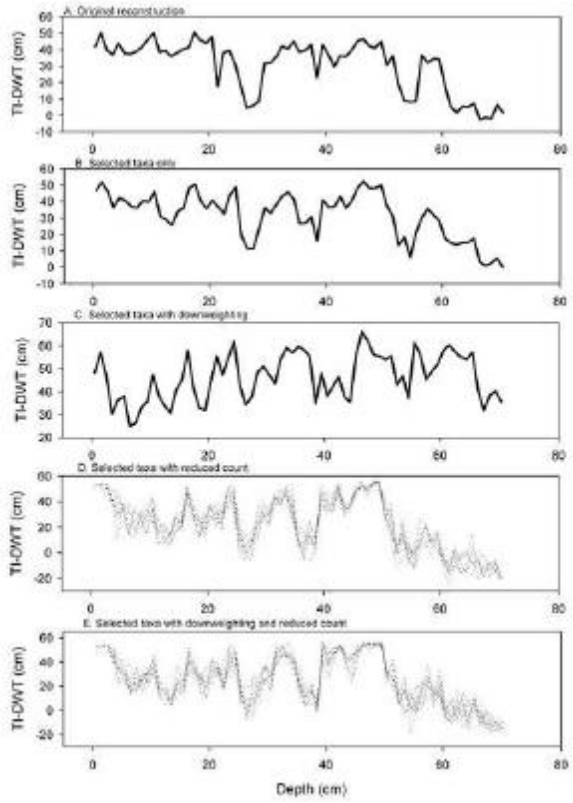
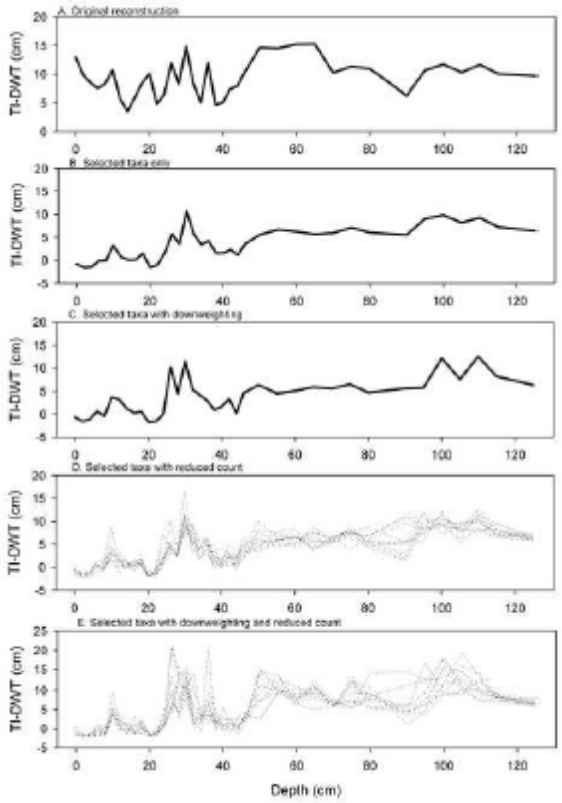


Fig. 5. Simulation results for Jelenia Wyspa (Poland), see fig. 4 for details.



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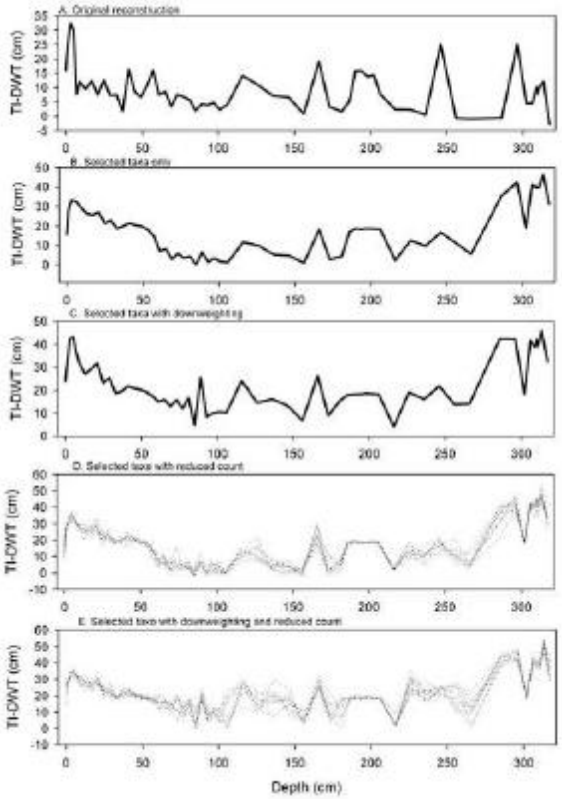
326 Fig. 6. Simulation results for Praz-Rodet (Poland), see fig. 4 for details.


A. Original reconstruction
B. Selected taxa only
C. Selected taxa with downweighting
D. Selected taxa with reduced count
E. Selected taxa with downweighting and reduced count
TI-DWT (cm)
Depth (cm)

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